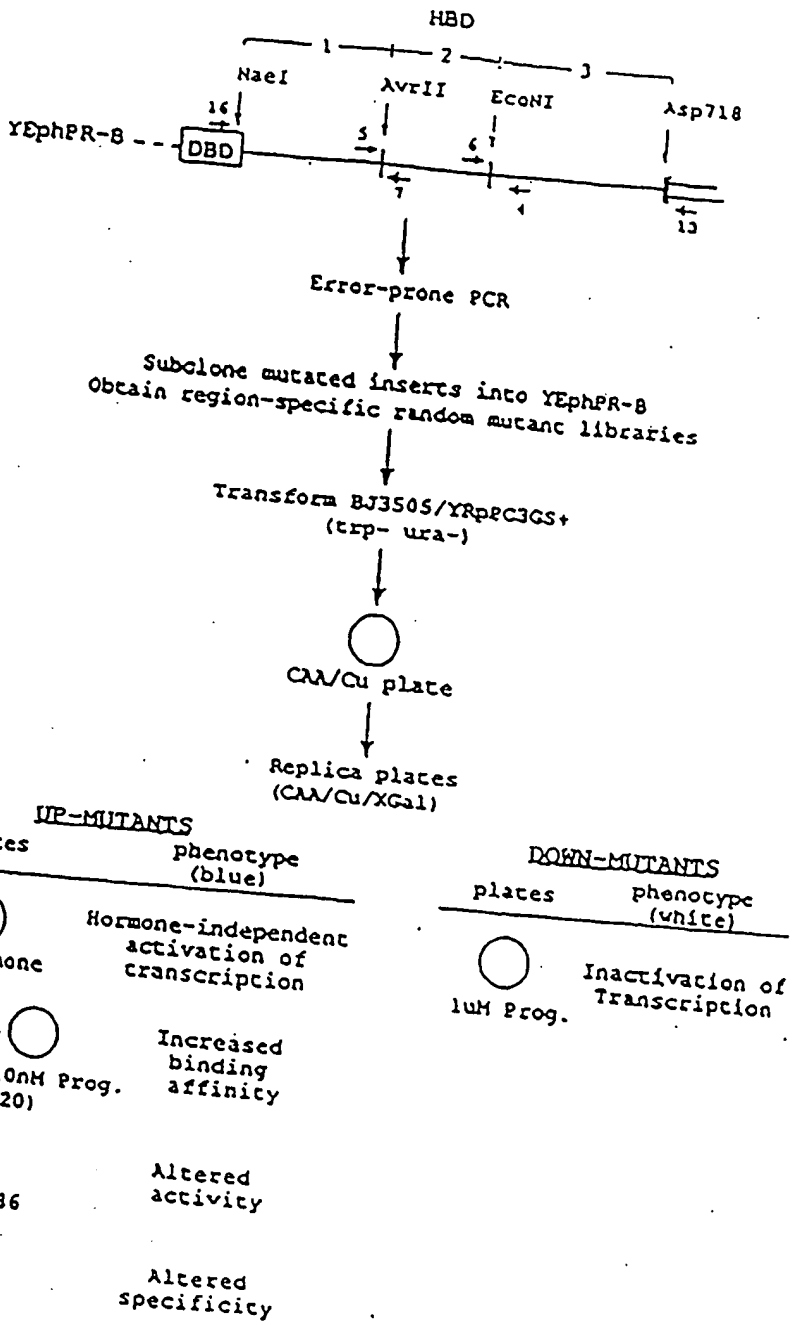

HBD
1
2
3
NaeI
AvrII
EcoNI
Asp718
16
YEphPR-B
DBD
5
6
7
4
13
Error-prone PCR
Subclone mutated inserts into YEphPR-B
Obtain region-specific random mutanc libraries
Transform BJ3505/YRpPC3GS+
(trp- ura-)
CAA/Cu plate
Replica plates
(CAA/Cu/XGal)
UP-MUTANTS
plates
phenotype
(blue)
no hormone
Hormone-independent
activation of
transcription
1uM Prog. 10nM Prog.
(or R5020)
Increased
binding
affinity
1uM RU486
Altered
activity
1uM Dex
Altered
specificity
DOWN-MUTANTS
plates
phenotype
(white)
1uM Prog.
Inactivation of
Transcription

Figure 1

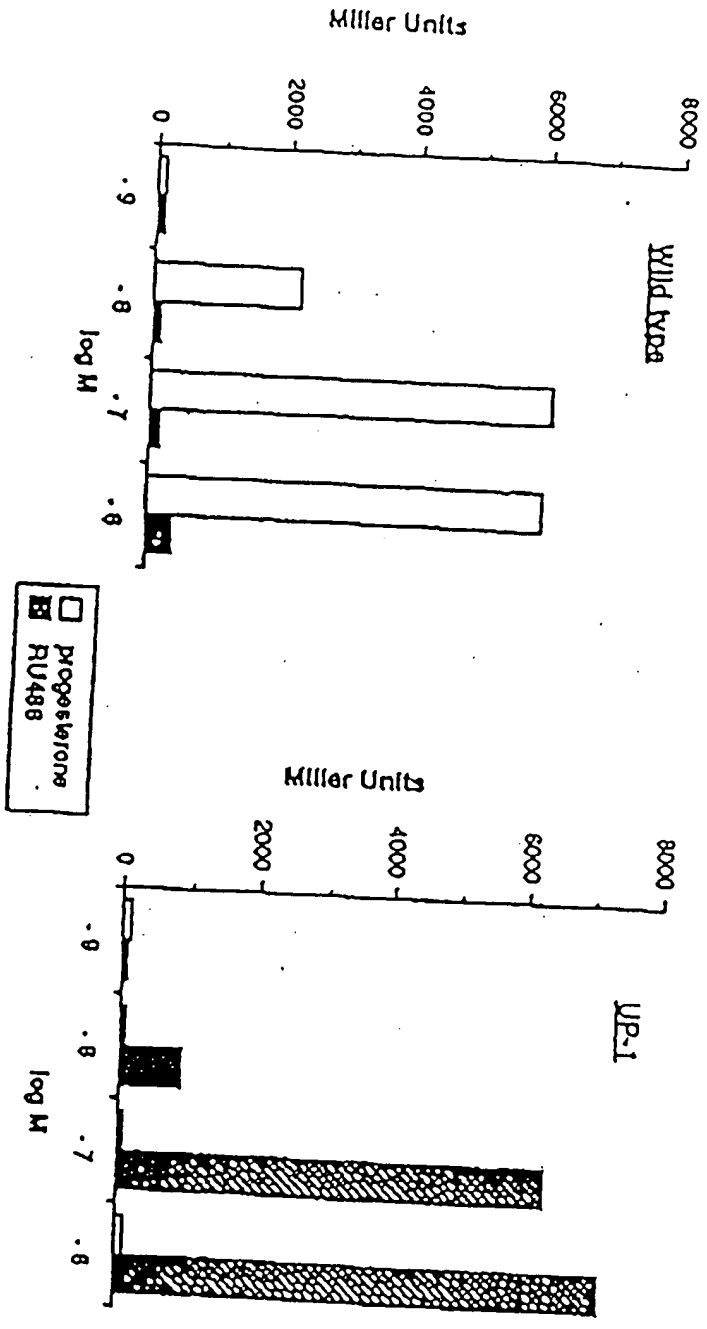

A
Miller Units
0
2000
4000
6000
8000
-9
-8
-7
-6
log M
Wild type
Progesterone
RU486
Miller Units
0
2000
4000
6000
8000
-9
-8
-7
-8
log M
UP-1

Figure 2A

B

DNA sequence:

2636
WT ..AAC TTG CAT GAT CTT GTC AAA CAA CTT CAT CTG TAC TGC TTG..

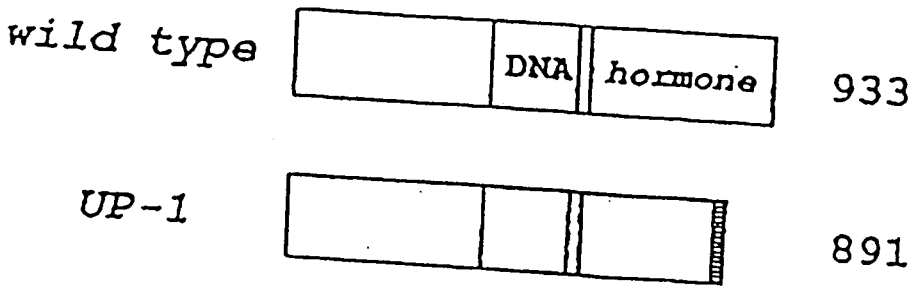
UP-1..AAT TGC ATG ATC TTG TCA AAC AAC TTC ATC TGT ACT GCT TGA

Protein sequence:

879
WT ..Asn Leu His Asp Leu Val Lys Gln Leu His Leu Tyr 891 Cys Leu..

UP-1..Asn Cys Met Ile Leu Ser Asn Asn Phe Ile Cys Thr Ala

Figure 2B



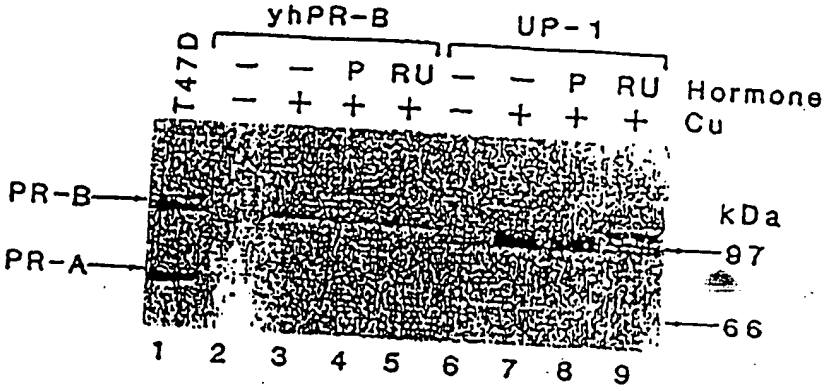


Figure 3

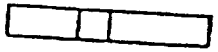
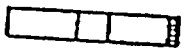
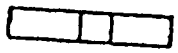
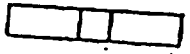
hPR Constructs	Transcriptional Activity (Miller Units)			Specific Binding (nM)	
	-	P	RU	P	RU
YEphPR-B (933) 	86	6200	586	1.0	1.3
UP-1 	286	466	8050	0.02	1.6
YEphPR-B879 	166	242	5900	0.04	1.8
YEphPR-B891 	243	226	6175	0.03	1.6

Figure 4

0959013.102897

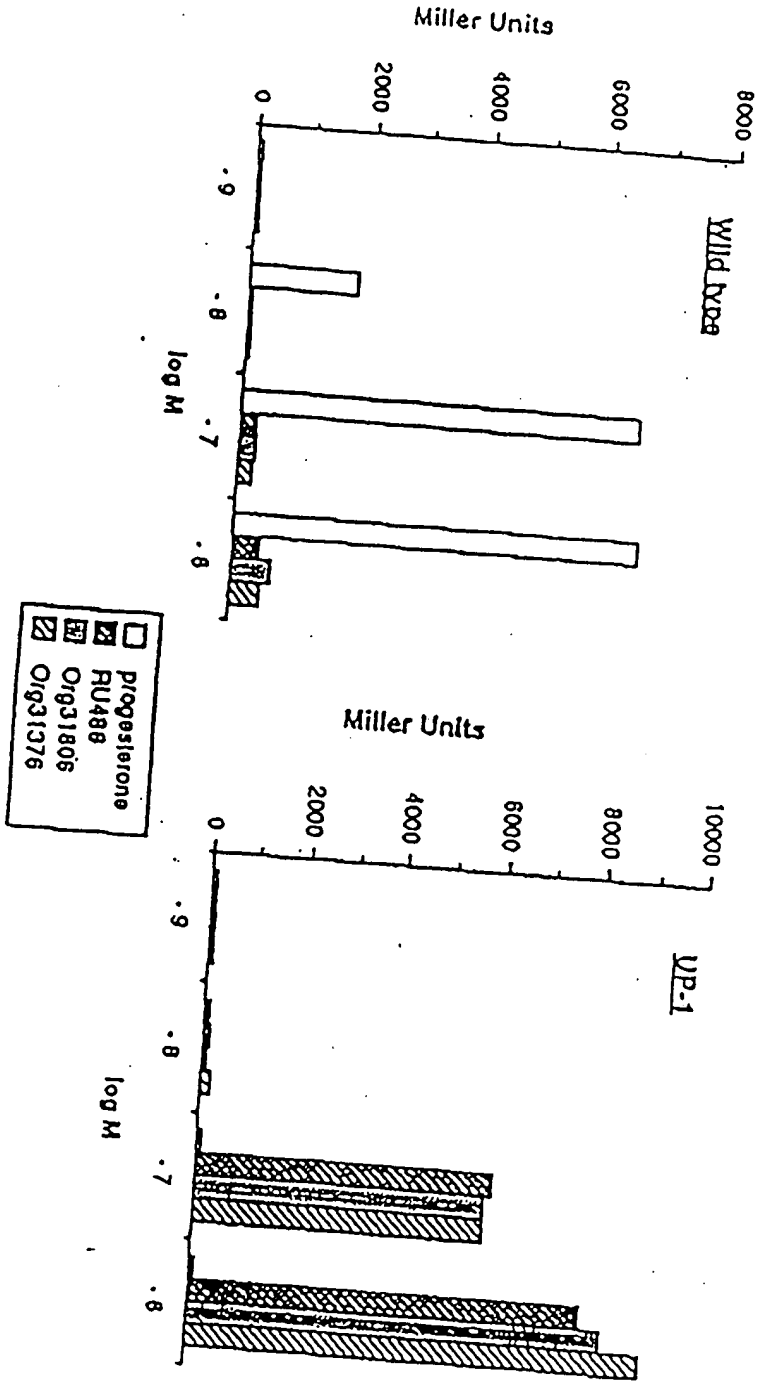

A
Miller Units
0
2000
4000
6000
8000
Wild type
log M
-6
-8
-7
-8
progesterone
RU486
Org31806
Org31376
Miller Units
0
2000
4000
8000
8000
10000
UP-1
log M
-6
-8
-7
-8

Figure 5A

B

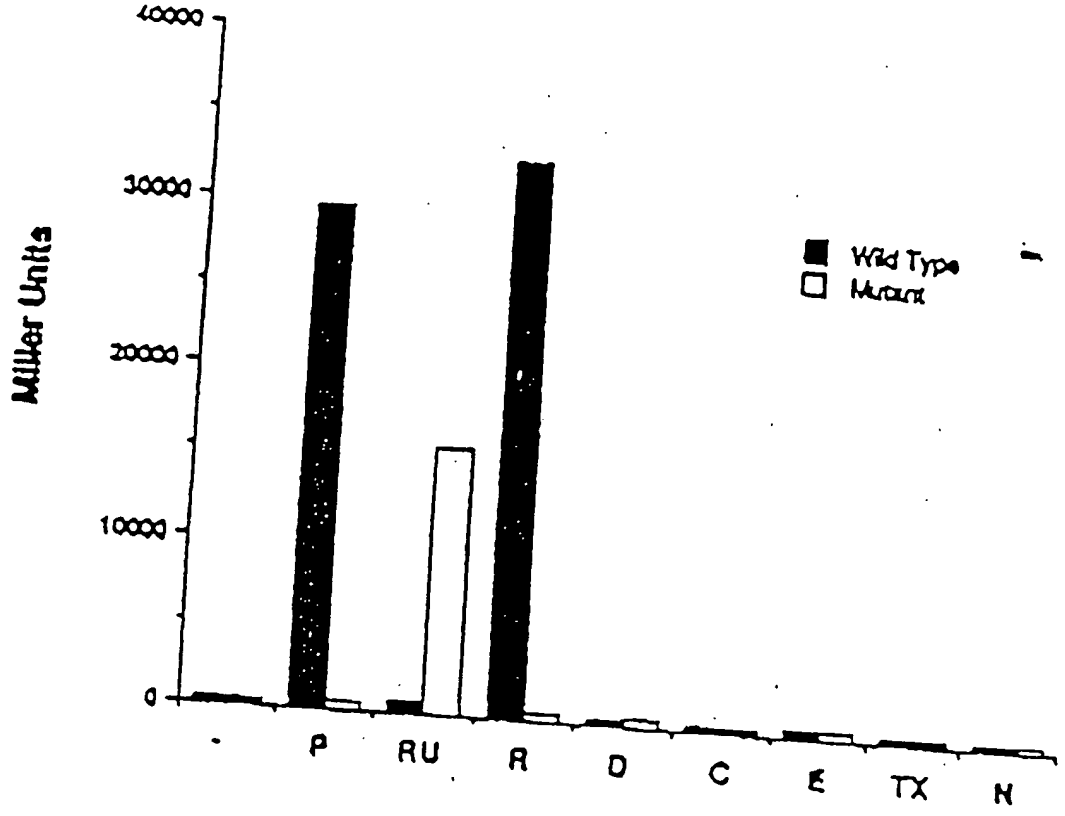

40000
30000
20000
10000
0
Miller Units
Wild Type
Mutant
P
RU
R
D
C
E
TX
N

Figure 5B

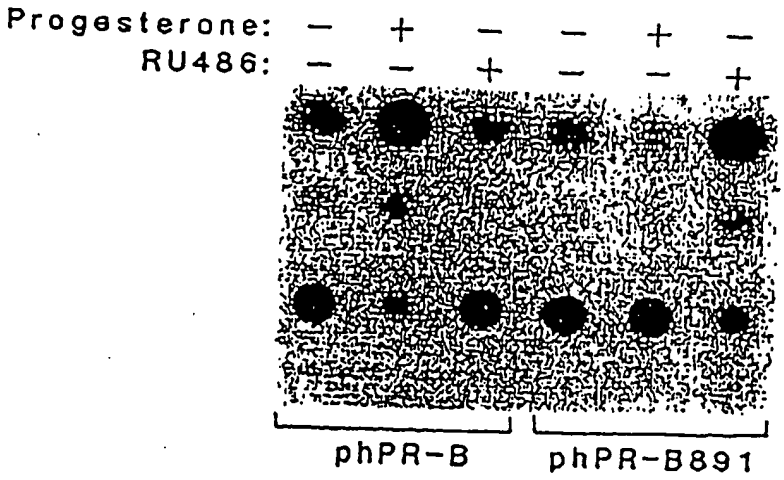


Figure 6A

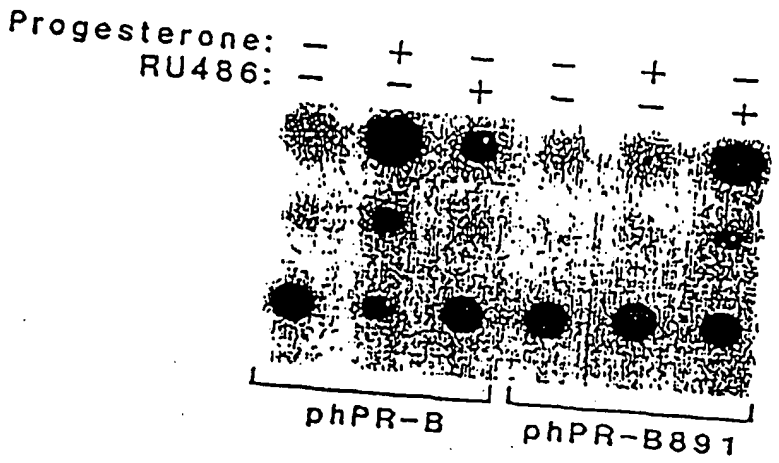


Figure 6B

GR PR Fusion Constructs

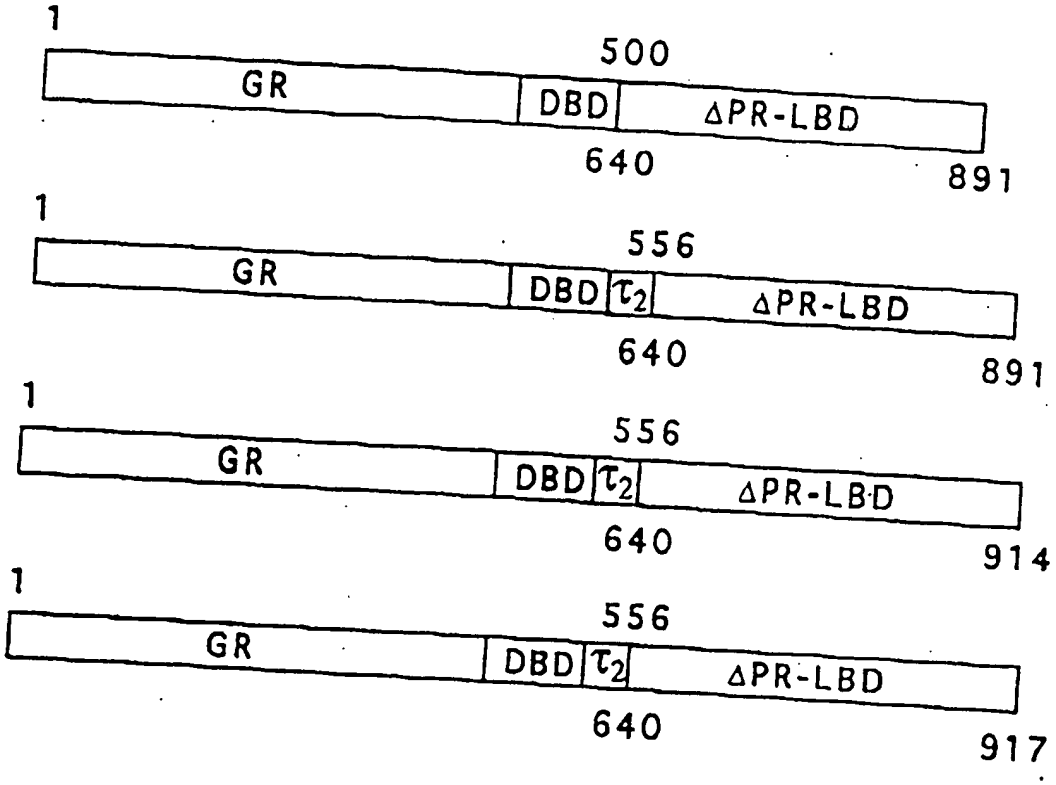
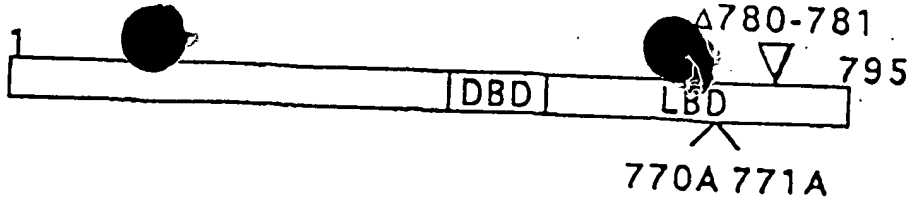
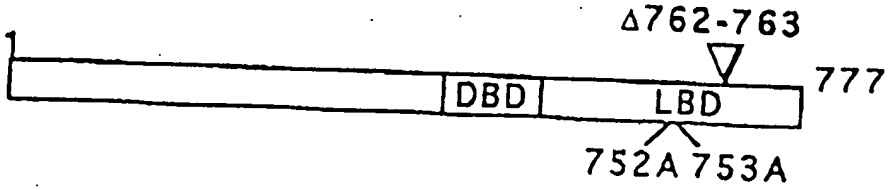


Figure 7

960505.P3



HUMAN GR MUTANTS



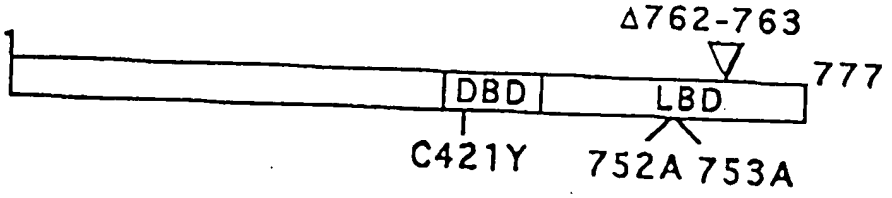


Figure 8

DOCKET ALARM

FIGURE 9

ORIGIN

```
   1 ctagagtcga cctgcagccc aagctctcga gggatcctga gaacttcagg gtgagtttgg
  61 ggacccttga ttgttctttc tttttcgcta ttgtaaaatt catgttatat ggaggggtca
 121 aagttttcag ggtgttgttt agaatgggaa gatgtcccct gtatcaccat ggaccctcat
 181 gataatttgg tttctttcac tttctactct gttgacaacc attgtctcct cttatttttct
 241 tttcattttc tgtaactttt tcgttaaact ttagcttgca tttgtaacga atttttaaat
 301 tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt tttcaaggca
 361 atcagggtat attatattgt acttcagcac agttttagag aacaattgtt ataattaaat
 421 gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt cttattggta
 481 gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa tgatatacac
 541 tgtttgagat gaggataaaa tactctgagt ccaaaccggg ccccctctgct aaccatgttc
 601 atgccttctt ctttttccta cagctcctgg gcaacgtgct ggttgttgtg ctgtctcatc
 661 attttggcaa agaattcact cctcaggtgc aggctgccta tcagaaggtg gtggctggtg
 721 tggccaatgc cctggctcac aaataccact gagatctttt tccctctgcc aaaaattatg
 781 gggacatcat gaagcccctt gagcatctga cttctggcta ataaaggaaa tttatttttca
 841 ttgcaatagt gtgttggaat tttttgtgtc tctcactcgg aaggacatat gggagggcaa
 901 atcatttaaa acatcagaat gagtatttgg tttagagttt ggcaacatat gccatatgct
 961 ggctgccatg aacaaaggtg gctataaaga ggtcatcagt atatgaaaca gccccctgct
1021 gtccattcct tattccatag aaaagccttg acttgaggtt agatttttt tatattttgt
1081 tttgtgttat ttttttcttt aacatcccta aaattttcct tacatgtttt actagccaga
1141 tttttcctcc tctcctgact actcccagtc atagctgtcc ctcttctctt atgaactcga
1201 ggagcttttt gcaaaagcct aggcctccaa aaaagcctcc tcactacttc tggaatagct
1261 cagaggccga ggcggcctcg gcctctgcat aaataaaaaa aattagtcag ccatggggcg
1321 gagaatgggc ggaactgggc ggagttaggg gcgggatggg cggagttagg ggcgggacta
1381 tggttgctga ctaattgaga ctgcattaat gaatcggcca acgcgcgggg agaggcggtt
1441 tgcgtattgg gcgctcttcc gcttcctcgc tcactgactc gctgcgctcg gtcgttcggc
1501 tgcggcgagc ggtatcagct cactcaaagg cggtaatacg gttatccaca gaatcagggg
1561 ataacgcagg aaagaacatg tgagcaaaag gccagcaaaa ggccaggaac cgtaaaaagg
1621 ccgcgttgct ggcgtttttc cataggctcc gccccccctga cgagcatcac aaaaatcgac
1681 gctcaagtca gaggtggcga aacccgacag gactataaag ataccaggcg tttccccctg
1741 gaagctccct cgtgcgctct cctgttccga ccctgccgct taccggatac ctgtccgcct
1801 ttctcccttc gggaagcgtg gcgctttctc aatgctcacg ctgtaggtat ctcagttcgg
1861 tgtaggtcgt tcgctccaag ctgggctgtg tgcacgaacc ccccgttcag cccgaccgct
1921 gcgccttatc cggtaactat cgtcttgagt ccaacccggt aagacacgac ttatcgccac
1981 tggcagcagc cactggtaac aggattagca gagcgaggta tgtaggcggt gctacagagt
2041 tcttgaagtg gtggcctaac tacggctaca ctagaaggac agtatttggt atctgcgctc
2101 tgctgaagcc agttaccttc ggaaaaagag ttggtagctc ttgatccggc aaacaaacca
2161 ccgctggtag cggtggtttt tttgtttgca agcagcagat tacgcgcaga aaaaaaggat
2281 gttaagggat tttggtcatg agattatcaa aaaggatctt cacctagatc cttttaaatt
2341 aaaaatgaag ttttaaatca atctaaagta tatatgagta aacttggtct gacagttacc
2401 aatgcttaat cagtgaggca cctatctcag cgatctgtct atttcgttca tccatagttg
2461 cctgactccc cgtcgtgtag ataactacga tacgggaggg cttaccatct ggccccagtg
2521 ctgcaatgat accgcgagac ccacgctcac cggctccaga tttatcagca ataaaccagc
2581 cagccggaag ggccgagcgc agaagtggtc ctgcaacttt atccgcctcc atccagtcta
2641 ttaattgttg ccgggaagct agagtaagta gttcgccagt taatagtttg cgcaacgttg
2701 ttgccattgc tacaggcatc gtggtgtcac gctcgtcgtt tggtatggct tcattcagct
2761 ccggttccca acgatcaagg cgagttacat gatcccccat gttgtgcaaa aaagcggtta
2821 gctccttcgg tcctccgatc gttgtcagaa gtaagttggc cgcagtgtta tcactcatgg
2881 ttatggcagc actgcataat tctcttactg tcatgccatc cgtaagatgc ttttctgtga
2941 ctggtgagta ctcaaccaag tcattctgag aatagtgtat gcggcgaccg agttgctctt
3001 gcccggcgtc aatacgggat aataccgcgc cacatagcag aactttaaaa gtgctcatca
3061 ttggaaaacg ttcttcgggg cgaaaactct caaggatctt accgctgttg agatccagtt
3121 cgatgtaacc cactcgtgca cccaactgat cttcagcatc ttttactttc accagcgttt
3181 ctgggtgagc aaaaacagga aggcaaaatg ccgcaaaaaa gggaataagg gcgacacgga
3241 aatgttgaat actcatactc ttccttttc aatattattg aagcatttat cagggttatt
3301 gtctcatgag cggatacata tttgaatgta tttagaaaaa taaacaaata ggggttccgc
3361 gcacatttcc ccgaaaagtg ccacctgacg tctaagaaac cattattatc atgacattaa
```

09/530,044 PCT/US98/23053

```
3421 cctataaaaa taggcgtatc acgaggccct ttcgtcttca agctgcctcg cgcgtttcgg
3481 tgatgacggt gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta
3541 agcggatgcc gggagcagac aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg
3601 gggcgcagcc atgacccagt cacgtagcga tagcggagtt ggcttaacta tgcggcatca
3661 gagcagattg tactgagagt gcaccatatc gacgctctcc cttatgcgac tcctgcatta
3721 ggaagcagcc cagtagtagg ttgaggccgt tgagcaccgc cgccgcaagg aatggtgctg
3781 gcttatcgaa attaatcgac tcactatagg gagacccgaa ttcgagctcg ccccgttaca
3841 taacttacgg taaatggccc gcctggctga ccgcccaacg acccccgccc attgacgtca
3901 ataatgacgt atgttcccat agtaacgcca atagggactt tccattgacg tcaatgggtg
3961 gagtatttac ggtaaactgc ccacttggca gtacatcaag tgtatcatat gccaagtacg
4021 ccccctattg acgtcaatga cggtaaatgg cccgcctggc attatgccca gtacatgacc
4081 ttatgggact ttcctacttg gcagtacatc tacgtattag tcatcgctat taccatggtg
4141 atgcggtttt ggcagtacat caatgggcgt ggatagcggt ttgactcacg gggatttcca
4201 agtctccacc ccattgacgt caatgggagt ttgttttggc accaaaatca acgggacttt
4261 ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg gcggtaggcg tgtacggtgg
4321 gaggtctata taagcagagc tcgtttagtg aaccgtcaga tcgcctggag acgccatcca
4381 cgctgttttg acctccatag aagacaccgg gaccgatcca gcctccgcgg gatcttggtg
4441 gcgtgaaact cccgcacctc ttcggccagc gccttgtaga agcgcgtatg gcttcgtggg
4501 gatcccccaa agaatcctta gctccccctg gtagagacga agtccctggc agtttgcttg
4561 gccaagggag ggggagcgta atggactttt ataaaagcct gaggggagga gctacagtca
4621 aggtttctgc atcttcgccc tcagtggctg ctgcttctca ggcagattcc aagcagcaga
4681 ggattctcct tgatttctcg aaaggctcca caagcaatgt gcagcagcga cagcagcagc
4741 agcagcagca gcagcagcag cagcagcagc agcagcagca gcagcagcca ggcttatcca
4801 aagccgtttc actgtccatg gggctgtata tgggagagac agaaacaaaa gtgatgggga
4861 atgacttggg ctacccacag cagggccaac ttggcctttc ctctggggaa acagactttc
4921 ggcttctgga agaaagcatt gcaaacctca ataggtcgac cagcgttcca gagaacccca
4981 agagttcaac gtctgcaact gggtgtgcta ccccgacaga gaaggagttt cccaaaactc
5041 actcggatgc atcttcagaa cagcaaaatc gaaaaagcca gaccggcacc aacggaggca
5101 gtgtgaaatt gtatcccaca gaccaaagca cctttgacct cttgaaggat ttggagtttt
5161 ccgctgggtc cccaagtaaa gacacaaacg agagtccctg gagatcagat ctgttgatag
5221 atgaaaactt gctttctcct ttggcgggag aagatgatcc attccttctc gaagggaaca
5281 cgaatgagga ttgtaagcct cttattttac cggacactaa acctaaaatt aaggatactg
5341 gagatacaat cttatcaagt cccagcagtg tggcactacc ccaagtgaaa acagaaaaag
5401 atgatttcat tgaactttgc acccccgggg taattaagca agagaaactg ggcccagttt
5461 attgtcaggc aagcttttct gggacaaata taattggtaa taaaatgtct gccatttctg
5521 ttcatggtgt gagtacctct ggaggacaga tgtaccacta tgacatgaat acagcatccc
5581 tttctcagca gcaggatcag aagcctgttt ttaatgtcat tccaccaatt cctgttggtt
5641 ctgaaaactg gaataggtgc caaggctccg gagaggacag cctgacttcc ttgggggctc
5701 tgaacttccc aggccggtca gtgttttcta atgggtactc aagccctgga atgagaccag
5761 atgtaagctc tcctccatcc agctcgtcag cagccacggg accacctccc aagctctgcc
5821 tggtgtgctc cgatgaagct tcaggatgtc attacggggt gctgacatgt ggaagctgca
5881 aagtattctt taaaagagca gtggaaggac agcacaatta cctttgtgct ggaagaaacg
5941 attgcatcat tgataaaatt cgaaggaaaa actgcccagc atgccgctat cggaaatgtc
6001 ttcaggctgg aatgaacctt gaagctcgaa aaacaaagaa aaaaatcaaa gggattcagc
6061 aagccactgc aggagtctca caagacactt cggaaaatcc taacaaaaca atagttcctg
6121 cagcattacc acagctcacc cctaccttgg tgtcactgct ggaggtgatt gaacccg
```

DRAFT - 2005-04-20 09:30:00

11

57

6178

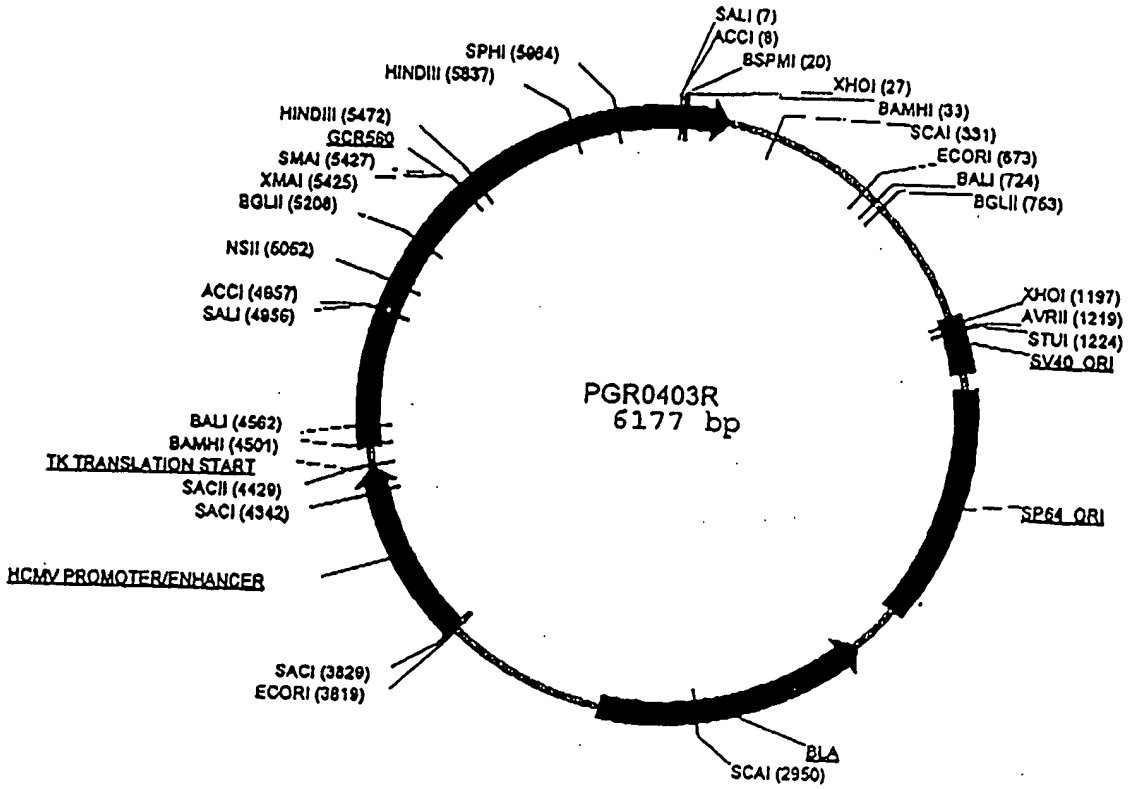


Figure 10

09505041.1 = 09505041.1

receptor	treatment	pg CAT protein induced	Normalized to control
wild-type hGR	control	0.4	1
	DEX	64.4	161
	RU	1.4	3.5
GRPR fusion	control	0.9	1
	DEX	0.6	0.7
	RU	5.4	6
wild-type rat GR	control	2.2	1
	DEX	26.4	12
	RU	6.3	2.9
CS1.CD	control	2.2	1
	DEX	1.8	0.8
	RU	29.6	13.5

Figure 11

0895013.102897

Glucocorticomimetic Receptor

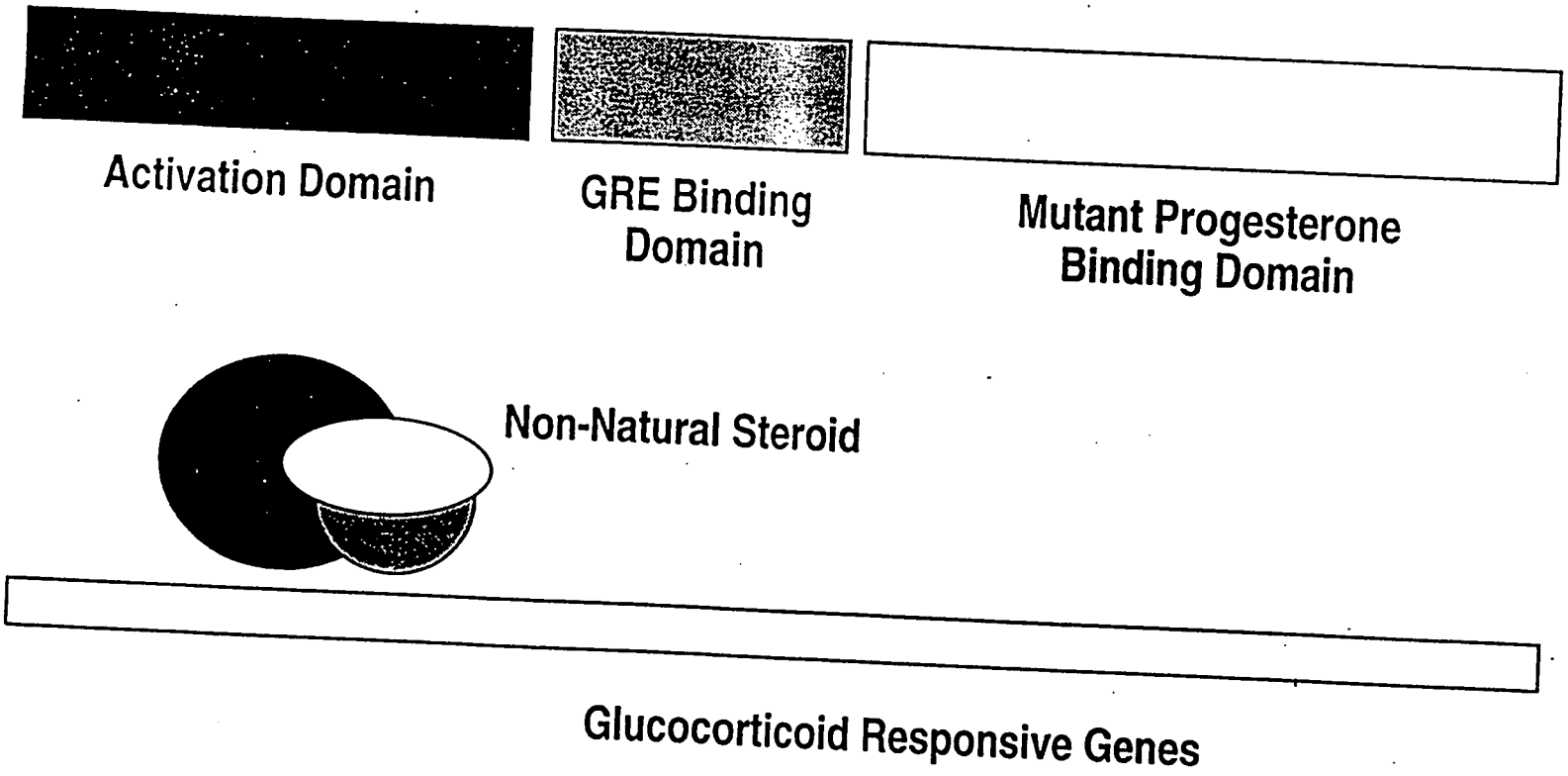


Figure 12

08959013.102897

Gene Switch

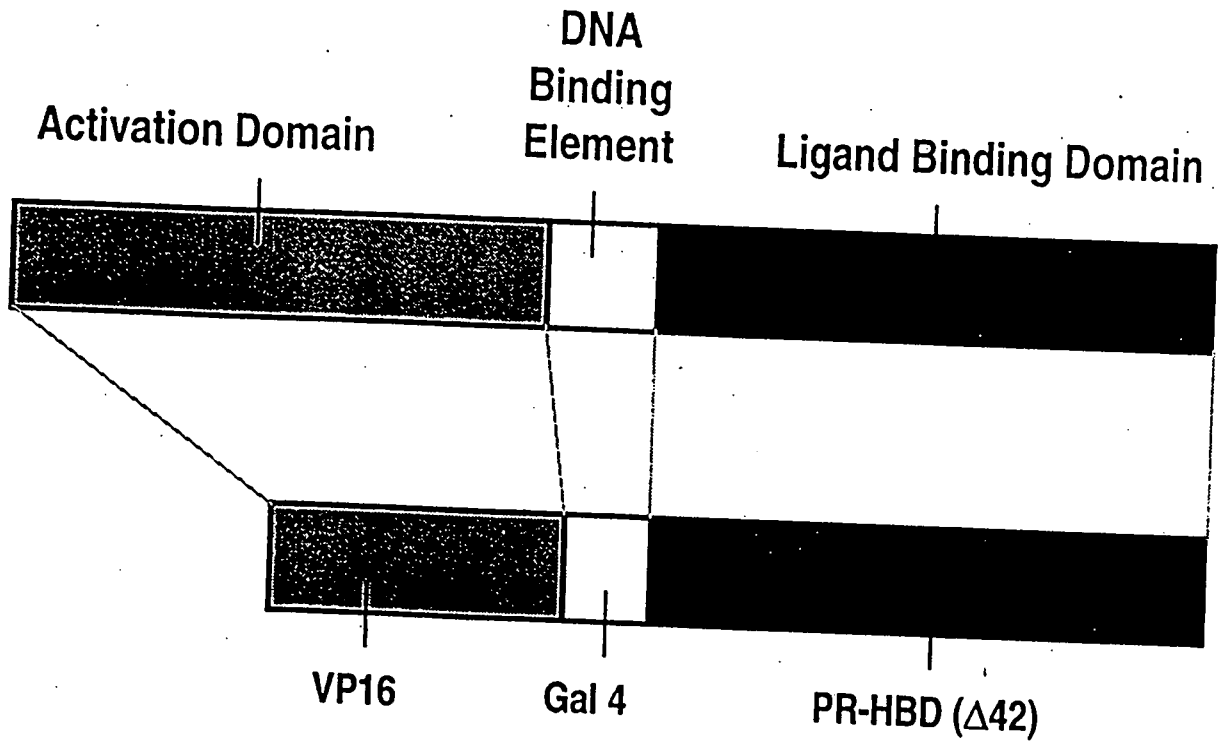
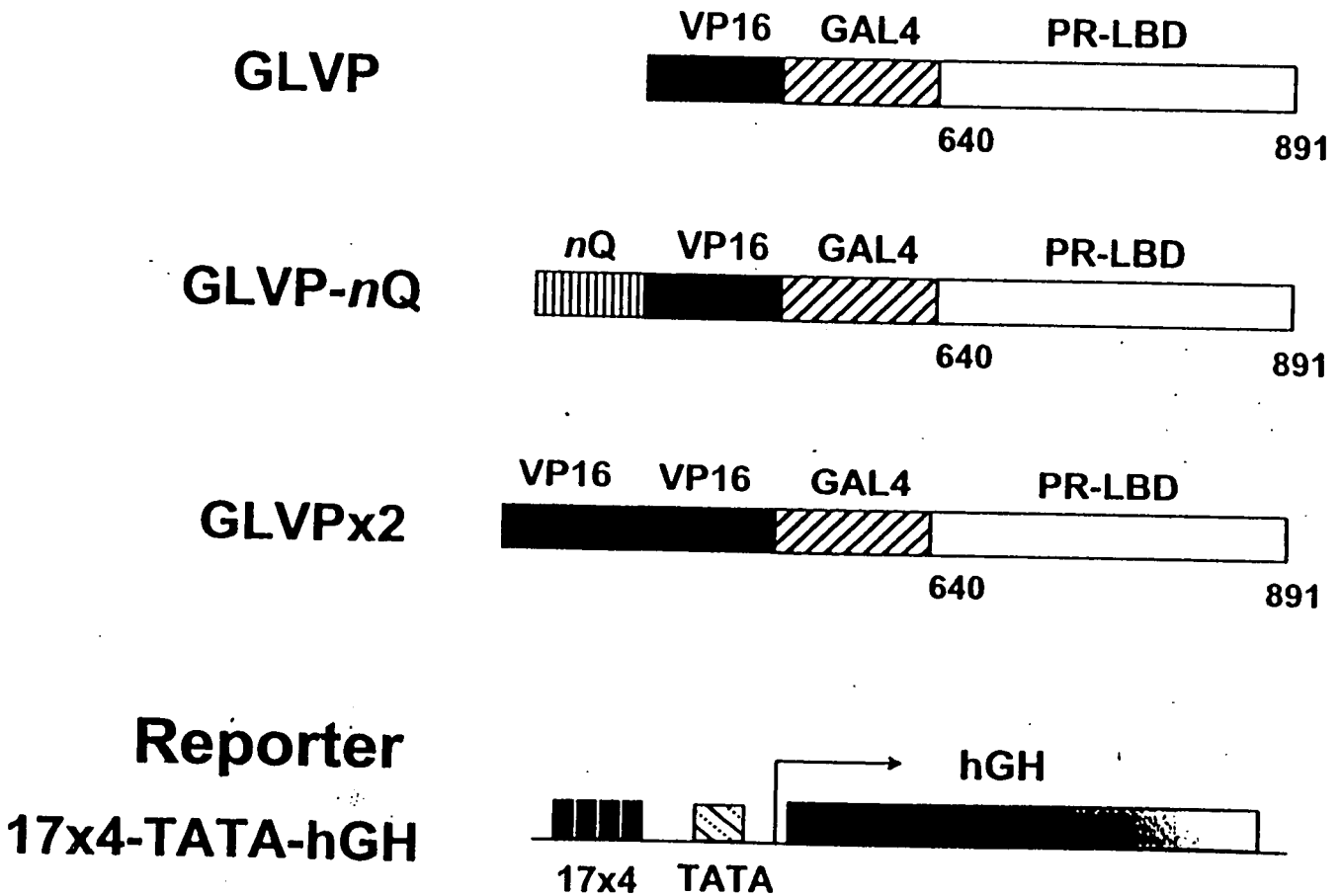


Figure 13

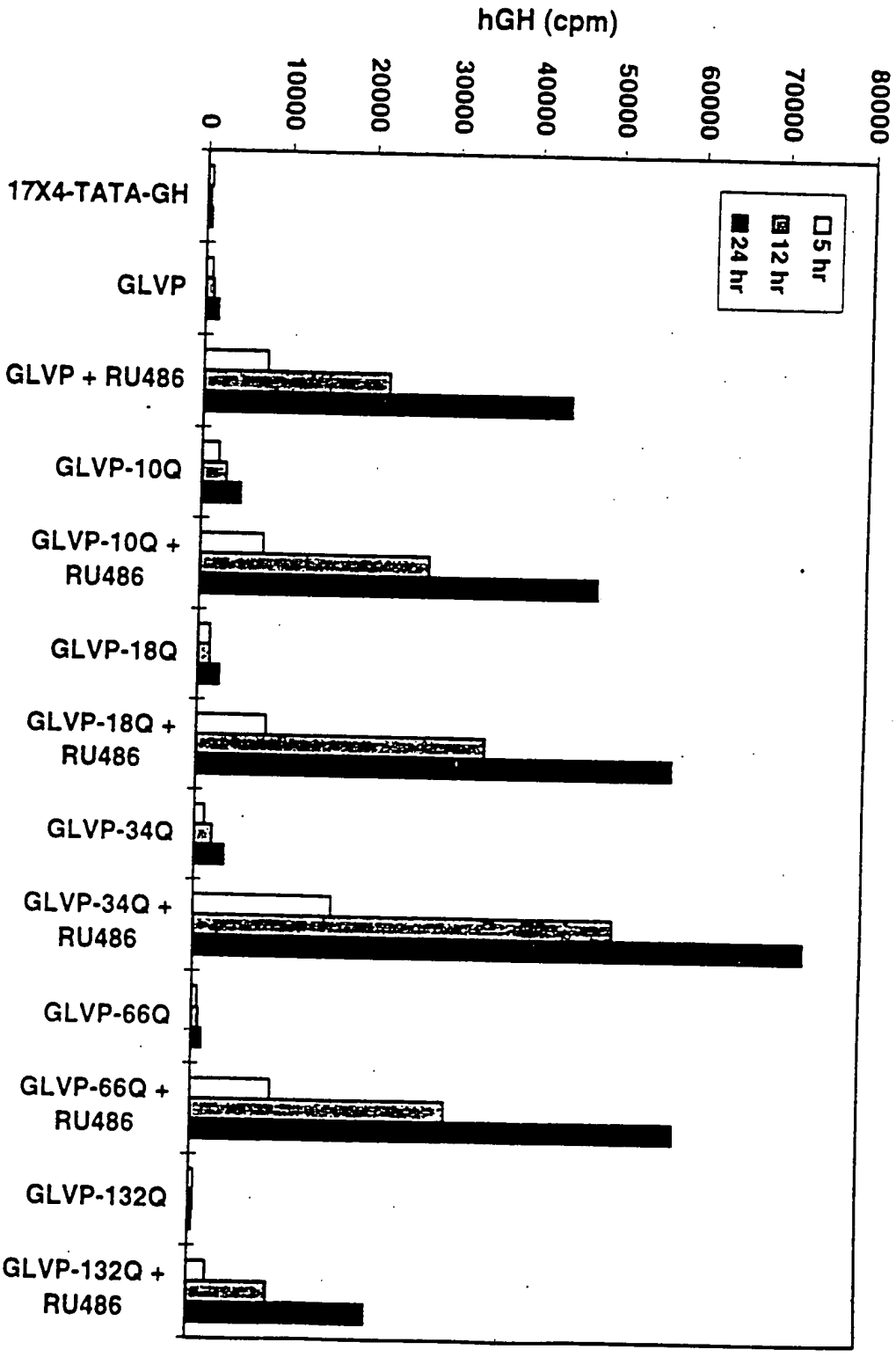
0959013.102897

FIG. 14



08959013.102897

FIG. 15



WO 95/27044 PCT/US95/03850

FIG. 16

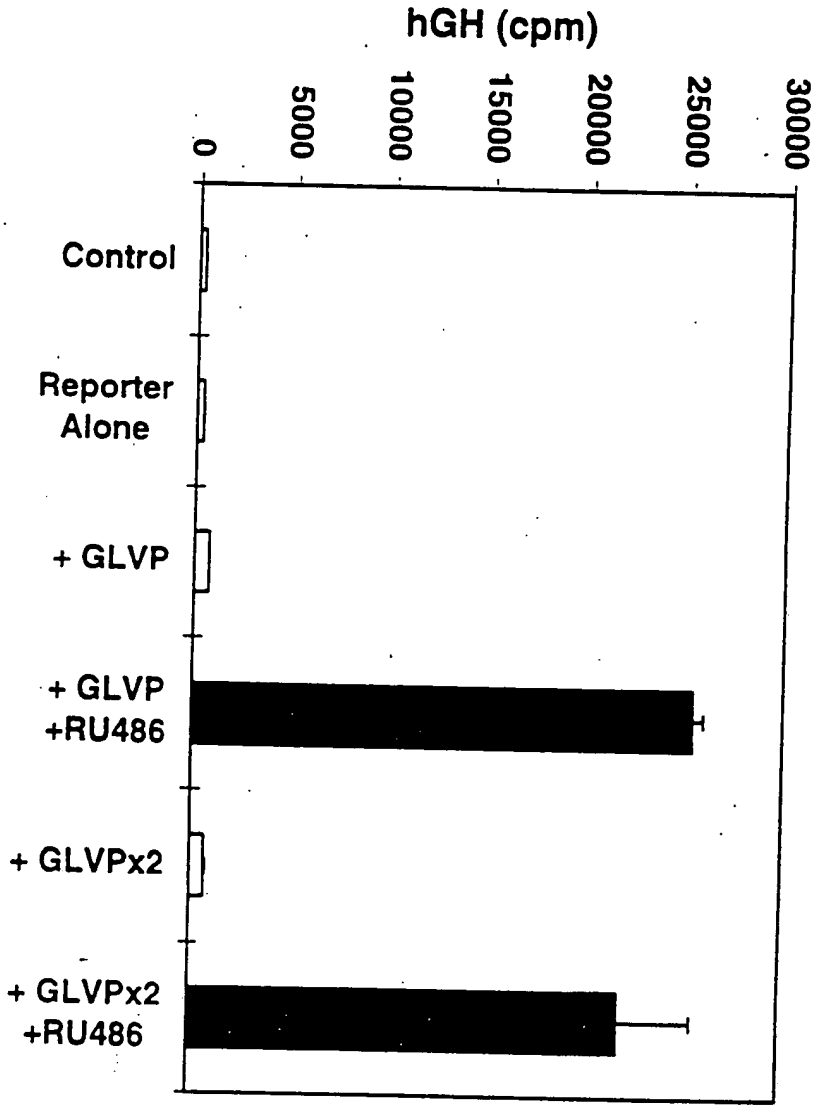
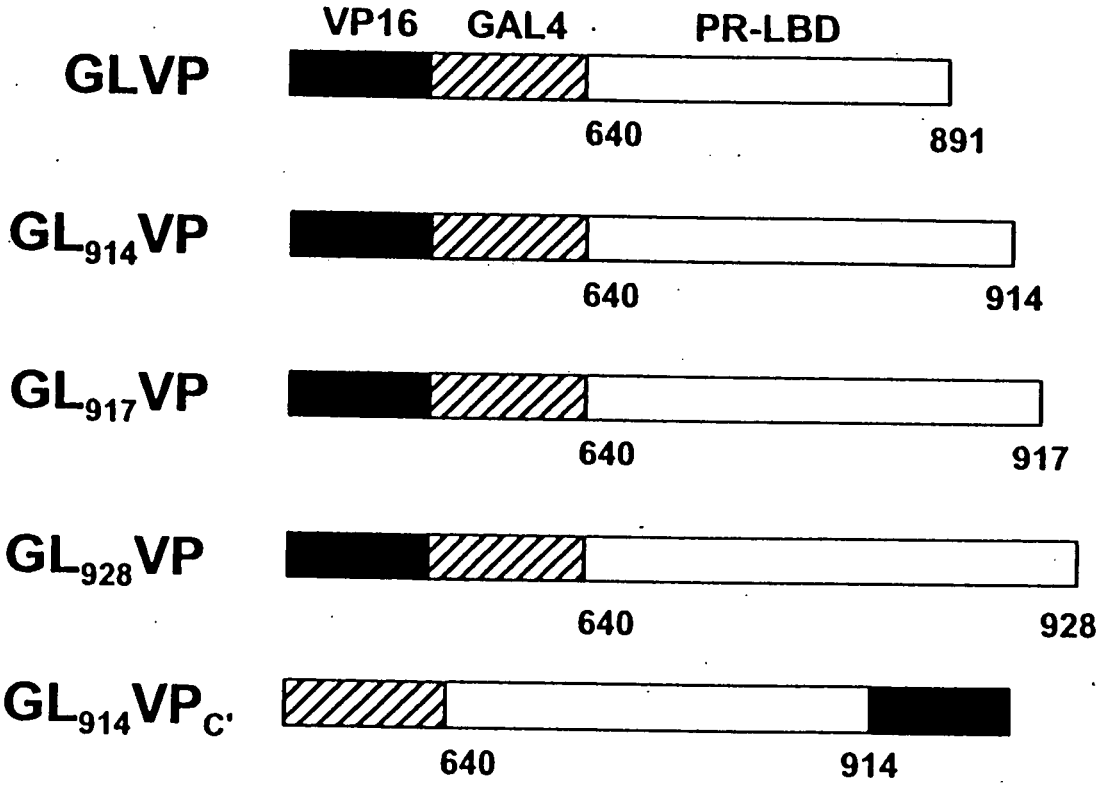
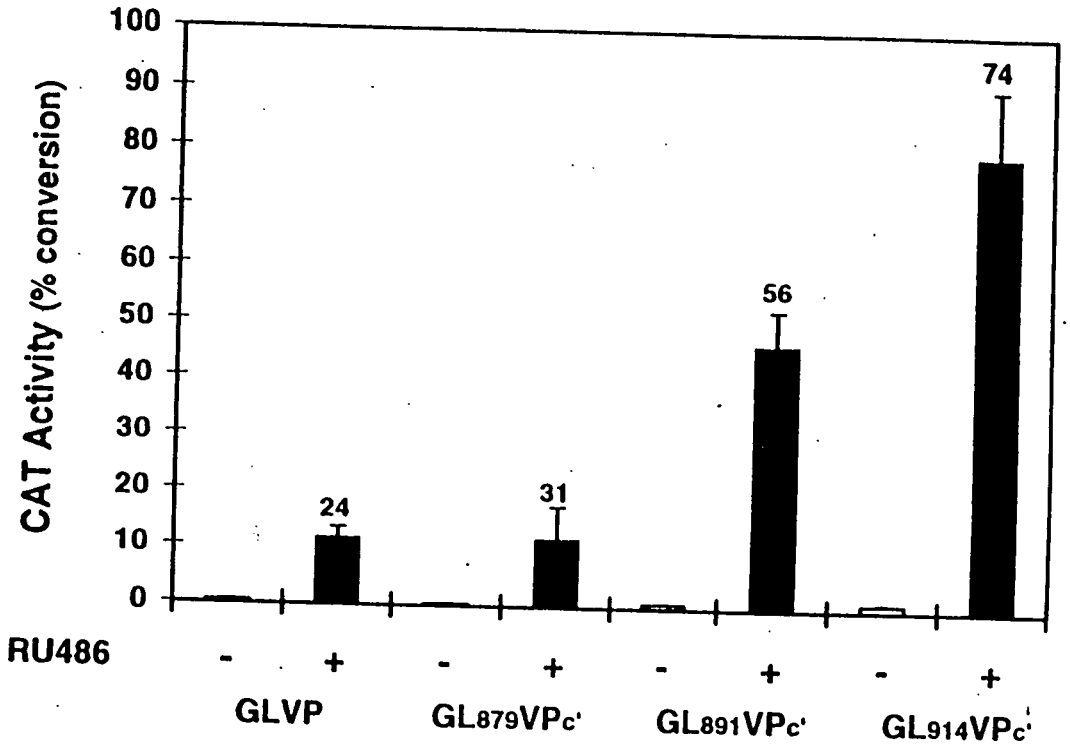

hGH (cpm)
0
5000
10000
15000
20000
25000
30000
Control
Reporter Alone
+ GLVP
+ GLVP +RU486
+ GLVPx2
+ GLVPx2 +RU486

FIG. 17



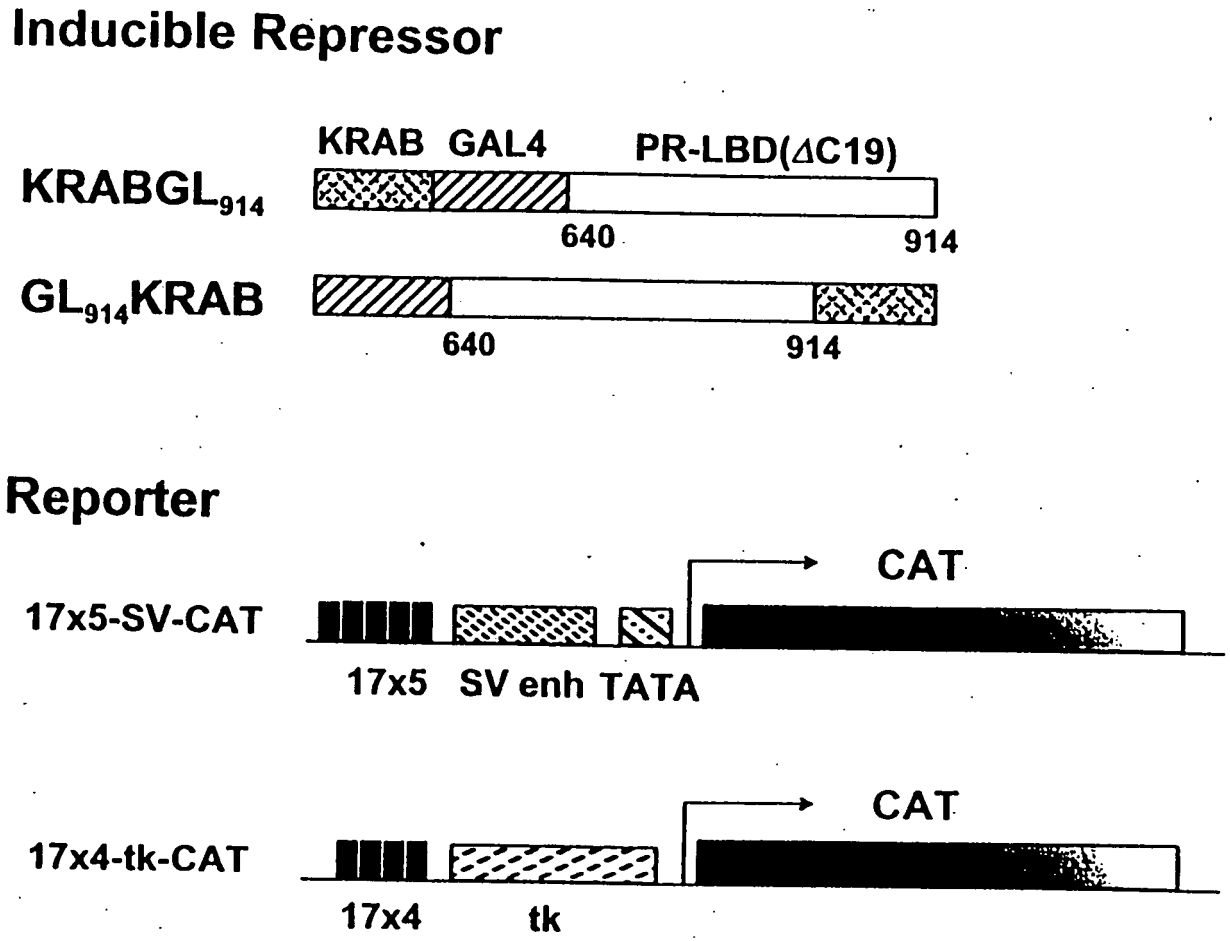
08959013 .102897

FIG. 18



08959013 .102697

FIG. 19



08959013 .102897